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(54) Title: MODIFIED ANTIBODY FRAGMENTS

(57) **Abstract:** The present invention provides an antibody Fab or Fab' fragment to which at least one effector molecule is attached characterized in that the heavy chain in the fragment is not covalently bonded to the light chain and both the interchain cysteine of C_L and the interchain cysteine of C_H1 have been replaced with another amino acid.



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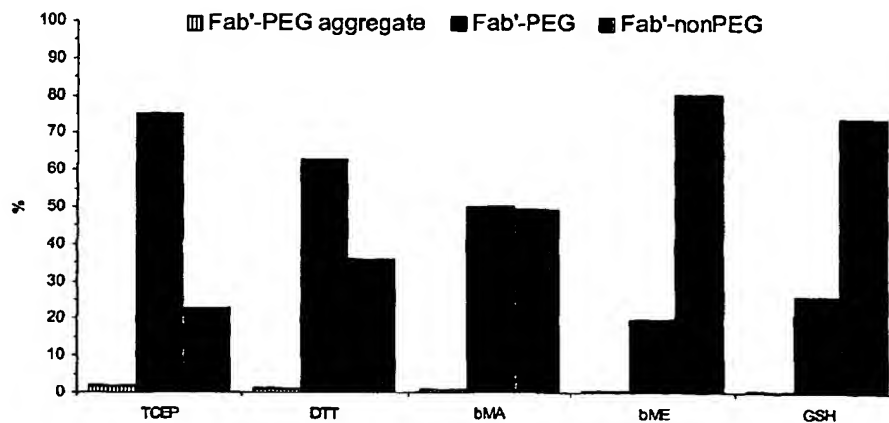
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(57) Abstract: The present invention provides an antibody Fab or Fab' fragment to which at least one effector molecule is attached characterized in that the heavy chain in the fragment is not covalently bonded to the light chain and both the interchain cysteine of C_L and the interchain cysteine of C_H1 have been replaced with another amino acid.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, MEDLINE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/15549 A (CELLTECH THERAPEUTICS LTD ; HUMPHREYS DAVID PAUL (GB)) 1 April 1999 (1999-04-01)	1-19, 27-30
Y	page 24, line 8 - line 24; claims; examples; table 1	1-30
X	WO 97/36932 A (KALLAND TERJE ; PHARMACIA & UPJOHN AB (SE); ANTONSSON PER (SE); HANSSO) 9 October 1997 (1997-10-09)	1-7, 27, 28, 30
Y	page 24, line 18 - page 25, line 6; claims; examples	1-30
P,X	EP 1 419 786 A (BRACCO IMAGING SPA) 19 May 2004 (2004-05-19)	1, 6-9, 30
P,Y	page 4, line 33 - page 7, line 24; claims; examples	1-30
	-/-	

☒ Further documents are listed in the continuation of box C.

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUMPHREYS D P ET AL: "Formation of dimeric Fabs in Escherichia coli: effect of hinge size and isotype, presence of interchain disulphide bond, Fab' expression levels, tail piece sequences and growth conditions" JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 209, no. 2, 1997, pages 193-202, XP004103797 ISSN: 0022-1759	1-15,17, 18,27, 28,30
Y	abstract; materials and methods; results; discussion;	1-30
X	HUMPHREYS D P ET AL: "Efficient site specific removal of a C-terminal FLAG fusion from a Fab' using copper(II) ion catalysed protein cleavage" PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 12, no. 2, February 1999 (1999-02), pages 179-184, XP000882102 ISSN: 0269-2139	1-11,13, 15,17, 27,28,30
Y	abstract; materials and methods; results;	1-30
Y	BURMEISTER GETZ E ET AL: "Comparison between the sulfhydryl reductants tris(2-carboxyethyl)phosphine and dithiothreitol for use in protein biochemistry" ANALYTICAL BIOCHEMISTRY, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 273, 15 August 1999 (1999-08-15), pages 73-80, XP000946001 ISSN: 0003-2697 abstract; discussion;	19-26

INTERNATIONAL SEARCH REPORT

PCT/GB2004/002870

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
- a. type of material
- ☒ a sequence listing
- ☐ table(s) related to the sequence listing
- b. format of material
- ☒ in written format
- ☒ in computer readable form
- c. time of filing/furnishing
- ☐ contained in the international application as filed
- ☐ filed together with the international application in computer readable form
- ☒ furnished subsequently to this Authority for the purpose of search
2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

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Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9915549	A	01-04-1999	AT 257160 T AU 9174398 A DE 69820885 D1 DE 69820885 T2 EP 1015495 A2 ES 2212340 T3 WO 9915549 A2 JP 2001517423 T US 6642356 B1	15-01-2004 12-04-1999 05-02-2004 16-12-2004 05-07-2000 16-07-2004 01-04-1999 09-10-2001 04-11-2003
WO 9736932	A	09-10-1997	AU 707827 B2 AU 2525197 A BR 9702179 A CA 2222757 A1 CZ 9703712 A3 EP 0835266 A1 ID 16512 A JP 2002502363 T NO 975435 A NZ 329145 A PL 323626 A1 RU 2198895 C2 WO 9736932 A1 TW 517061 B US 2003092894 A1 US 6514498 B1 ZA 9702492 A	22-07-1999 22-10-1997 16-03-1999 09-10-1997 13-05-1998 15-04-1998 02-10-1997 22-01-2002 29-01-1998 23-06-2000 14-04-1998 20-02-2003 09-10-1997 11-01-2003 15-05-2003 04-02-2003 02-10-1997
EP 1419786	A	19-05-2004	EP 1419786 A1 WO 2004043492 A1	19-05-2004 27-05-2004